

STUDIES ON THE EFFECTS OF IRRADIATION OF CELLULAR PARTICULATES. IV. THE TIME SEQUENCE OF PHOSPHORYLATION CHANGES *IN VIVO*¹

HENRY T. YOST, JR., ROBERT M. GLICKMAN AND LAURENCE H. BECK

Department of Biology, Amherst College, Amherst, Mass.

Previous work has demonstrated that mitochondrial oxidative phosphorylation is more sensitive to uncoupling by ionizing radiation when the mitochondria are exposed *in vivo* than when they are irradiated *in vitro* (van Bekkum, 1957; Yost and Robson, 1959; Benjamin and Yost, 1960). These findings are consistent with the reports of the effects of irradiation on the phosphorylating mechanism by a number of other authors and have led to the hypothesis that the action of radiation in producing the *in vivo* effect is indirect and probably moderated by radiation-induced alterations of the hormonal balance of the organism.

The main purpose of the studies reported in this paper was to determine the time sequence of inactivation and recovery of the phosphorylating mechanism. The data presented by Benjamin and Yost (1960) were insufficient to permit conclusions about the time of onset and the time of repair of damage. This is particularly evident since van Bekkum (1957) had reported activation as early as eight hours post-irradiation and Noyes and Smith (1959) reported inactivation of a short-term nature at one hour post-irradiation. Consequently the pattern of inactivation was investigated, using our techniques, to determine whether the effect studied in this laboratory was the same as that studied by others. Furthermore, it was possible to correlate the damage to the phosphorylating mechanism with damage to radio-sensitive cells and tissues for which the time sequence is well established, particularly the spleen (Metcalf, Blandaw and Barnett, 1950) and the circulating lymphocytes (Jacobson, 1954).

In the second place, studies of the time sequence of inactivation can be used to further test the hypothesis of indirect action of radiation on the phosphorylating mechanism. Experiments were performed in which the time of onset and the time of repair of damage were determined for total-body and partial-body exposure. For example, rats whose heads were shielded were compared with those whose entire body had been exposed, on the proposition that, if the effect was largely moderated by hormonal changes originating in the pituitary, head-shielded rats would show a reduced response. Such experiments have the additional value of providing evidence to assess whether "resistance" to irradiation is a function of initial resistance to damage or of the rate of regeneration of damaged cells and tissues.

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Finally, since previous studies have often given results in which the P:O values are relatively low, it seemed wise to reinvestigate the problem with techniques which would insure "normal" P:O values for control animals. Recently, reports by Thomson and Rahman (1962) and Thomson (1964) have suggested that the observed inactivation of phosphorylation by irradiation is an artifact of assay procedures which give low P:O values. Such an objection does not apply to this work, since the data presented have been collected in such a way that low P:O values are avoided. Although the technical problems raised by Thomson will be discussed elsewhere, it is significant to point out that the studies done in the present paper show that the times at which the assays were done with spleen mitochondria (Thomson, 1964) were such that no effect of irradiation would have been expected. Therefore, it is hoped that the publication of the time-sequence data in this paper will make possible better experimental design for investigation of this rather complex indirect effect of irradiation, in the future.

MATERIALS AND METHODS

All experiments were carried out with young, male, albino rats weighing from 200 to 250 grams. Rats of the Sprague-Dawley or Wistar lines were used exclusively. With the exception of those rats assayed soon after irradiation, all rats were anesthetized prior to exposure with 0.04–0.05 mg. Nembutal per gram of body weight. At the times of assay, there was no residual effect of the anesthetic on phosphorylation. Those rats assayed up to eight hours after exposure were irradiated without anesthetic, in a small cage permitting little freedom of motion.

Shielding of the anterior abdomen was accomplished by covering the region (from the lower extremity of the rib cage to beneath the stomach) with two inches of lead plate. (This method gave the same result as shielding the exteriorized spleen (Benjamin and Yost, 1960) and resulted in far fewer fatalities. However, since more than the spleen was shielded, this procedure may decrease the effectiveness of the radiation.) Head-shielding was performed in a similar manner, with the head removed to the edge of the field, as well as covered by lead. Measurements with a Victoreen dosimeter indicate that this method permits about 10% of the delivered dose to reach the shielded organs, since Co^{60} γ -rays are difficult to eliminate entirely. The rats were irradiated singly in all cases, and during the period between treatment and assay, they were fed Purina Lab Chow and kept supplied with water. In order to assure maximum uniformity, large numbers of rats were irradiated successively, caged together, and selected randomly for post-irradiation assay. Control rats were processed in exactly the same manner with the sole exception of exposure to radiation. All radiation was delivered from a 220-curie Co^{60} source, filtered with $\frac{1}{2}$ inch of Lucite, at an intensity of 90 r/min. Since there is a slight lack of uniformity of dose resulting from the thickness of the animals, the dose was calculated for a plane passing through the pituitary and spleen of the rats.

One hour before sacrificing a group of rats, lymphocyte counts were made. Blood was obtained by cutting off one-eighth inch of the tail. The first drop was discarded in all cases where the state of the animal permitted. Cell counts were

made at a dilution of 1:20, using a dilution fluid of 1% acetic acid and gentian violet. Replicate counts done with control blood samples indicated an error in counting of 10%.

The rats were sacrificed by a blow on the head and the spleens removed. Small sections of spleen tissue were taken from all experimental animals of any one run. (Since samples taken from the tip and from the mid-part of the control spleens indicated that the tissue was homogeneous, and since the small size of spleens from irradiated rats made it inadvisable to sacrifice much tissue for histological examination, sections were taken routinely near the tip of each spleen.) The tissue was fixed in Bouin's fluid for 12 to 15 hours, washed and dehydrated with alcohol, and embedded in paraffin. Sections were cut at 10 μ and stained with hematoxylin-eosin stains. Sections from spleens of the different animals within any one experiment were kept separate and compared, to determine the amount of variation existing in animals receiving the same treatment.

The bulk of the spleen tissue (remaining after the above procedure) was pooled from two or three rats and homogenized in cold, isotonic sucrose solution (5 ml. per spleen) containing 0.005 *M* disodium versenate. Mitochondria were isolated from the homogenate by differential centrifugation (Schneider, 1948), with the particulates collected at 9000 *g* and washed once. Routinely, mitochondria derived from control animals were suspended in 0.9 ml. sucrose-Versene and those derived from irradiated animals were suspended in 0.5 ml. for assay. The unequal dilutions were made necessary by the lower yield of mitochondria from irradiated animals (as measured by O.D.). If the preparation is too dilute, generation of ATP does not occur, regardless of the source of the mitochondria. After irradiation, the spleen of the rat undergoes extensive change and the yield of mitochondria is greatly reduced. To maintain an adequate concentration of mitochondria, it is necessary to use an *apparently* more concentrated preparation from the irradiated spleens, otherwise phosphate uptake does not occur. To obtain reproducible results, very concentrated preparations of mitochondria were used throughout the experiments. (It is important to note that the mitochondrial preparation cannot be too concentrated; the addition of excess amounts of mitochondria, over a wide range, makes no difference to the P:O ratio. It is only essential that a certain minimal concentration be maintained.) The technique used in this paper insures that P:O ratios greater than zero will be obtained in all experimental cases; however, it is important to note that we are measuring the ability of *surviving* mitochondria to phosphorylate and possibly over-estimating the ability of the cells from irradiated animals to generate ATP, since the number of mitochondria per cell may be greatly reduced (Detrick, Upham, Springsteen, McCandless and Haley, 1964).

Liver mitochondria were prepared by homogenizing approximately 2 grams of liver removed from a single rat. The yield of mitochondria from liver is very high, and consequently it was unnecessary to pool mitochondria. In the case of testis mitochondria, however, four testes were necessary (two rats) to provide a sufficiently large pellet of mitochondria to give even minimally acceptable P:O ratios. Thus, only in the case of liver mitochondria do the data represent findings derived from a single animal. Otherwise, any individual P:O value represents an "average" of the conditions in two or three rats.

TABLE I
Depression of spleen phosphorylation by 800 r, total-body.
Sprague-Dawley rats

Time after exposure	P:O ratio	(Range)	% Inactivation	No. runs	Histology*
0	1.9	(1.8—2.0)	0	3	normal, many large follicles
1 hr.	2.0	(1.8—2.4)	0	3	pycnotic nuclei
3 hrs.	1.8	(1.6—1.9)	5	3	
8 hrs.	1.4	(1.2—1.5)	26	5	follicles reduced, debris
24 hrs.	1.1	(0.7—1.6)	42	11	follicles absent
48 hrs.	0.9	(0.8—1.1)	53	4	follicles absent
3 days	1.2	(0.9—1.8)	37	8	follicles absent
5 days	1.3	(0.9—1.8)	32	8	
8 days	1.0	(0.5—1.7)	47	8	
14 days	1.0	(0.9—1.2)	47	4	follicles absent, new cells
30 days	1.4	(0.8—1.7)	26	4	follicles present but tiny
43 days	1.9	(1.8—2.0)	0	3	follicles regenerating
Control (ave.)	1.9	(1.3—2.4)	—	64	

* Refer to text for description.

The efficiency of respiratory energy conversion was measured by the P:O ratio, using the technique of Hunter (1955). Oxygen uptake was measured in a Warburg respirometer at 25° C. Readings were taken for 20 to 30 minutes, after a 5-minute equilibration period; satisfactory P:O ratios are obtained from concentrated spleen preparations, so long as at least 6 micro-atoms of oxygen are consumed within that time period. Succinate was used as the substrate, and the incubation medium was the same as described previously (Yost and Robson, 1959). In all cases, 1/2 ml. of mitochondrial suspension was added to each flask. Estimation of the remaining inorganic phosphorus was carried out by the method of Lowry and Lopez (Glick, 1949). All phosphate tests were run in duplicate.

TABLE II
Depression of spleen phosphorylation by 800 r, total-body.
Wistar rats

Time after exposure	P:O ratio	(Range)	% Inactivation	No. runs	Histology*
24 hrs.	1.2	(1.1—1.2)	33	3	follicles absent
4 days	1.1	(0.9—1.2)	39	4	
5 days	1.0	(1.0—1.1)	44	3	
6 days	1.1	—	39	3	follicles absent, new cells?
7 days	1.5	(1.3—1.6)	17	4	
8 days	1.7	(1.6—1.8)	6	6	tiny follicles, when present
11 days	1.8	(1.7—1.9)	0	3	follicles regenerating
Control (ave.)	1.8	(1.6—2.3)	—	26	

* Refer to text for description.

TABLE III
*Depression of liver phosphorylation by 800 r, total-body.
 Sprague-Dawley rats*

Days after exposure	P:O ratio	(Range)	% Inactivation	No. runs
2	1.2	(0.8—1.4)	33	5
4	1.4	(1.3—1.4)	22	5
6	1.5	(1.3—1.6)	17	5
8	1.6	(1.4—1.7)	11	5
10	1.7	(1.6—1.8)	6	5
12	1.8	(1.7—1.8)	0	5
Control (ave.)	1.8	(1.7—2.1)	—	30

RESULTS

Phosphorylation

The data in Tables I to VI are presented as average P:O values from all determinations at any particular time. Although individual control and experimental animals were processed simultaneously in each run, the amount of data presented in this paper is too extensive to permit individual experiments to be presented in tabular form, as has been done for other studies (Benjamin and Yost, 1960). For simplicity of presentation, control values from all experiments in any one table were averaged as a basis for comparison, rather than having the control average for each set of runs presented with the experimental. Inactivation is computed from the average P:O value for each time period compared to the overall average for the control. Values obtained in this way do not differ significantly from those obtained by presenting individual experiments (certainly the values for the inactivation are approximations at best). It is important to note that although there is a variation in the controls, whose range would appear to overlap that of the experimentals, at no time did experimental

TABLE IV
*Depression of testis phosphorylation by 800 r, total-body.
 Sprague-Dawley rats*

Days after exposure	P:O ratio	(Range)	% Inactivation	No. runs
2	0.7	(0.7—0.8)	42	5
4	0.8	(0.7—0.9)	33	5
6	0.9	(0.8—1.0)	25	5
8	0.9	(0.9—1.0)	25	5
10	0.9	(0.9—1.0)	25	5
12	1.0	(0.9—1.1)	17	5
14	1.1	(1.1—1.3)	8(?)	5
16	1.2	(1.1—1.3)	0	5
Control (ave.)	1.2	(1.1—1.3)	—	40

TABLE V

*Depression of spleen phosphorylation by 800 r, anterior-abdomen shielded.
Sprague-Dawley rats*

Time after exposure	P:O ratio	(Range)	% Inactivation	No. runs	Histology*
24 hrs.	0.6	(0.6—0.7)	68	3	follicles absent
48 hrs.	0.8	(0.4—1.1)	58	3	follicles absent
4 days	1.1	(0.9—1.3)	42	3	
6 days	1.6	(1.5—1.7)	16	5	follicles absent, new cells
8 days	1.8	(1.6—2.0)	5	6	follicles regenerating
11 days	1.9	(1.7—2.0)	0	3	follicles recovered
Control (ave.)	1.9	(1.6—2.0)	—	23	

* Refer to text for description.

P:O values in fact overlap the controls within individual experiments, until values of 5% inactivation (or lower) were reached. Thus, throughout the tables, the results of individual experiments are in agreement with the averaged data as presented.

One of the great difficulties in handling data of this type is in the development of a test for the significance of differences. Where the number of tests is suffi-

TABLE VI

*Depression of spleen phosphorylation by 800 r, partial-body.
Wistar rats*

Time after exposure	P:O ratio	(Range)	% Inactivation	No. runs	Histology*
Anterior-abdomen shielded					
24 hrs.	1.4	(1.3—1.5)	22	3	follicles absent
48 hrs.	1.2	(1.1—1.2)	33	3	follicles absent, new cells?
4 days	1.2	(1.0—1.3)	33	4	follicles regenerating
6 days	1.6	(1.2—1.7)	11	5	
8 days	1.9	(1.8—2.0)	0	3	follicles normal
Controls (ave.)	1.8	(1.5—2.0)	—	18	
Head Shielded					
24 hrs.	1.8	(1.6—2.0)	5	3	follicles reduced in size
5 days	1.8	(1.8—1.9)	5	3	follicles normal
8 days	1.9	(1.8—2.0)	0	2	normal
Controls (ave.)	1.9	(1.8—2.1)	—	8	

* Refer to text for description.

ciently great (a minimum of 6 and more favorably 10 runs), standard deviation values of approximately 0.15 are obtained. Thus, to get highly significant results it is necessary that the difference in P:O ratios between controls and experimentals be greater than 0.45. To be extremely cautious it is best to use the value 0.5. Thus, in Table I where the data are most extensive, it is clear that there is a significant difference between the depression which occurs at 24 hours, three days, five days, and 8 days, and the controls. By ordinary standard deviation analysis, however, no such significance could be demonstrated for many of the other lines of data. Therefore, taken individually such lines of data must be treated as suggestive of the pattern of inactivation in the organism, rather than as established differences in inactivation from the compared controls. On the other hand, since the pattern remains unchanged, and since the experimental values at no time overlap the control values (so long as inactivation has occurred), it seems permissible to lump the data for statistical treatment by Chi-square analysis. When this is done the difference between experimentals and controls is highly significant ($P < 0.001$). Thus, the data presented in this paper clearly indicate that inactivation of oxidative phosphorylation occurs after exposure to radiation and, taken as a whole, indicate that this alteration in the phosphorylating mechanism requires a certain minimum time to be expressed and a certain minimum time to be repaired. Within any table, however, differences between neighboring lines of data are likely to lack significance and must be treated merely as a probable pattern to the extent that they correlate with other types of damage which are readily observed in the organism.

Table I shows that the inactivation of phosphorylation by total-body irradiation is not permanent. In the interpretation of these data, it is necessary to remember that those rats assayed at later times are those which have survived the effects of the radiation. Therefore, it is hard to tell how much of the restitution is attributable to recovery and how much to initial radiation resistance. Unfortunately, it is impossible to assay the same rat at two different times. The sampling error is made clear by the range of P:O values. Even at 24 hours post-irradiation, there was *one* Sprague-Dawley preparation which *approached* normal values. In general, the P:O values cluster around the mean, but there are always a few which fall at the extremes. Part of this variation is the result of the difficulties normally associated with P:O determinations, but the existence of rats within the population which show greater resistance is a factor which cannot be ignored. By 48 hours after irradiation, no rats sampled show even near-normal phosphorylation. The examination of several hundred cases in various experimental series has shown this to be a consistent effect. Thus, any initial resistance to radiation must be a matter of the first hours. In general, it is evident that recovery is a more important process than is resistance, insofar as phosphorylative ability is concerned. This is further borne out by the data obtained from histological examination (see below), which indicate that the rats respond uniformly to a given dose of radiation. Taking all the tables together, it is clear that there is no evidence for resistance to the initial effects of the radiation, either insofar as the phosphorylating mechanism is concerned or with regard to the histology of the spleen itself.

The data presented in Table II indicate that the Wistar line recovers from the initial effects of the radiation much more rapidly than the Sprague-Dawley line. These experiments were originally conducted on the basis of a report (Boche and Bishop, 1950) suggesting that the Wistar strain of rats is more radiation-resistant than the Sprague-Dawley strain. Therefore, it seemed possible that there would be a difference in the damage done to the phosphorylation system by equal doses of radiation. It is clear that the initial effects of the radiation in both strains are the same. However, it is also clear that under the conditions of this experiment the Wistar rats recover the ability to phosphorylate much more rapidly than do the Sprague-Dawley rats. Unfortunately, attempts to demonstrate a difference in the radiation resistance of the two strains, under the conditions existing at this laboratory, have not met with success. In the survival studies, the initial resistance of the Wistar rats is higher than that of the Sprague-Dawley; that is to say, more deaths occur in the early days with the Sprague-Dawley rats than with the Wistar rats. However, by the end of 30 days both strains show approxi-

TABLE VII
*Depression of liver phosphorylation by 800 r, anterior-abdomen shielded.
Sprague-Dawley rats*

Days after exposure	P:O ratio	(Range)	% Inactivation	No. runs
2	1.3	(1.0—1.5)	28	4
4	1.2	(1.0—1.3)	33	4
6	1.4	(1.3—1.5)	22	4
8	1.8	(1.6—2.0)	0	4
10	1.8	(1.7—1.8)	0	4
12	1.8	(1.7—1.9)	0	4
Control (ave.)	1.8	(1.7—2.0)	—	24

mately the same response to radiation. Consequently, it appears that the alteration in the phosphorylating mechanism cannot be correlated directly with survival. Comparison of Tables I and II makes it clear that the recovery pattern is not an artifact of selection procedures. The two lines consistently recover at different times and are, therefore, at different levels of phosphorylative ability at the same time.

The data presented in Tables III and IV indicate that the effect on phosphorylation is not limited to the spleen. In both the liver and the testis of Sprague-Dawley rats an initial depression of phosphorylation is found, which follows the pattern observed in spleen. Similarly, there is recovery, but the rate of recovery is considerably different in different organs. Thus, the liver recovers much more rapidly than the spleen or the testis, the testis being intermediate in its rate. The work of Benjamin and Yost (1960) has indicated that the action of γ -rays is modified by different glands in producing the effect on liver and on spleen. (The nature of the action on the testis is not certain at the present time.) It is clear that, whatever the cause of the initial damage, the major difference in response is in the recovery rate. The collected data of Tables I-IV suggest that there

is a widespread depression of phosphorylative ability in the entire body at 24 to 48 hours after exposure to radiation.

The data presented in Tables V, VI and VII demonstrate the efficacy of anterior abdominal shielding in accelerating the recovery process. The spleens of Sprague-Dawley rats recover their ability to phosphorylate long before they would without the shielding (compare with Table I). In the Wistar line, the *acceleration* of the recovery process is not as marked but is none the less evident. Similarly, the liver recovers only slightly more rapidly, but the difference is interesting since the liver, itself, is not shielded. It can be seen that the Wistar strain shows very little effect of exposure to radiation so long as the head is shielded. It must be remembered, in considering these data, that approximately 10% of the radiation reaches the shielded area in these experiments. At the present time, working with gamma radiation, it is not possible for us to refine our shielding procedures.

Histology

It has been known for some time that exposure to radiation causes profound changes in the "white pulp" (hereafter called follicles) of the spleen (Metcalf, Blandaw and Barnett, 1950). It seemed logical to investigate such histological changes at the same time that assays of the ability of the spleen cells to carry on oxidative phosphorylation were being made. It was hoped to use these well-established changes in tissue organization as an internal control for the effect on phosphorylation. Since the histological changes reported here are in no way different (except sampling time) from those previously described by others (Jacobson, 1954; Metcalf, Blandaw and Barnett, 1950), only a brief description of the typical cases is given. A short summary phrase is included in the tables for ease of comparison.

The first evidence of radiation damage is pronounced nuclear pycnosis of the lymphocyte tissue of the follicles at about one hour after exposure. By 8 hours after exposure, the follicles have been reduced greatly in size, with a marked accumulation of cellular debris. At the end of 24 hours, the follicles have been reduced to such an extent that there is little evident follicular material. Even the cells closely surrounding the arterioles at the center of the follicles show marked pycnosis. There is a change in the number of cells in the red pulp as well (Metcalf, Blandaw and Barnett, 1950), but these were not studied in detail in the present work, as all histological changes in the spleen seem to follow the more striking pattern of the follicles. After 24 hours, the pattern of recovery varies with the type of exposure, dose, age, etc. The descriptions which follow are in general agreement with those reported elsewhere except in the time-sequence, which varies as a result of such differences.

The spleens of the Sprague-Dawley rats show marked follicular growth by the 40th day. Although the spleens have not yet returned to normal, there is no doubt that recovery is extensive by this time. Pycnotic cells are rare and follicle size is about one-half that of the control animals. At 11 days post-irradiation, the follicles show some sign of recovery (the process is initiated), but there are far fewer follicles and none are more than one-tenth the size of the controls.

By 30 days after exposure, the size of some of the follicles has returned to normal, although the number of follicles is still reduced. It is significant that no follicles with normal histological appearance were found in these rats up to 20 days after exposure.

Shielding of the spleen (anterior abdomen) has little effect on the initial histological effect of the radiation. Degeneration of the splenic follicles is as drastic, in most cases, although in a few cases some small areas of the major follicles (near the arteriole) remain. (The nuclei of such surviving cells show marked pycnosis.) The rate of recovery is remarkable. By four days after exposure, the Wistar rats show extensive regeneration, with the larger follicles approaching the normal condition; and by eight days after exposure, these rats have apparently recovered fully. Much the same is true of the Sprague-Dawley rats, although they were somewhat delayed when compared with the Wistar line.

The Wistar rats were studied after exposure with the head shielded. The initial histological effects were reduced but were evident nonetheless. It is clear

TABLE VIII
Lymphocyte counts after 800 r, total-body or partial-body exposure.
Sprague-Dawley and Wistar rats

Time after exposure	Total-body S-D	$\bar{c}_c^*$	Anterior-abdomen shielded S-D	$\bar{c}_c^*$	Total-body W	$\bar{c}_c^*$	W Head shielded	$\bar{c}_c^*$
24 hrs.	3350	26	3930	31	4400	35	4030	33
3 days	—	—	—	—	3230	25	7200	57
4 days	4120	32	6400	50	—	—	—	—
5 days	—	—	—	—	7000	55	9770	77
7 days	—	—	6480	51	—	—	—	—
8 days	4670	37	7570	60	9000	71	16120	127
10 days	4170	33	9000	71	—	—	—	—
12 days	4800	38	10000	79	10000	79	18220	143
14 days	**	—	13200	104	—	—	—	—

* Based on average control value of 12700/mm³.

** Extremely variable.

that follicular changes are induced even under these conditions. However, recovery is complete by five days post-irradiation, in all cases, and at no time is the damage as extensive as when the head is exposed.

The histological changes reported above are associated largely (although not entirely) with changes in the lymphocyte population. Since the spleen represents the largest mass of lymphocyte tissue in the body, it seemed advisable to attempt to correlate the changes in spleen organization and physiology with changes in the circulating lymphocyte population. The results of cell counts are presented in Table VIII. Because of the variability of the counts (the standard error of some counts was as high at 1000), the data can be taken only as a guide to the general pattern of effect and recovery. However, the depression in lymphocyte number follows the same pattern observed for phosphorylation and tissue damage. Where comparable, these results are in agreement with those of others (Jacobson, 1954).

DISCUSSION

The results presented in this paper clearly demonstrate that oxidative phosphorylation is uncoupled by exposure of rats to whole- and partial-body irradiation. The techniques employed in this work were such that the P:O values of control animals are in agreement with the theoretical value (2.0), with the exception of testis mitochondria. Therefore, the suggestion of Thomson (1964) that the effect of irradiation on oxidative phosphorylation is an artifact of preparation resulting in low P:O values for the controls as well as the experimentals would seem to be erroneous. The inability of Thomson to observe a depression of oxidative phosphorylation in spleen mitochondria can be attributed to the fact that his assays were made four hours after irradiation. As can be seen in Table I, the type of effect discussed in this paper can only be observed after 8 hours and cannot be clearly established until 24 hours after exposure to radiation. Although alterations in phosphorylative ability at shorter time periods have been reported (Noyes and Smith, 1959), such effects are generally of very short duration and are not the type discussed by van Bekkum (1957), Benjamin and Yost (1960) or in the body of this paper.

The data presented in this paper agree with previous findings (Jacobson, 1954; Swift, Taketa and Bond, 1954) that radiation-induced damage to the spleen is largely indirect. Both changes in oxidative phosphorylation and histological appearance are produced when radiation is delivered to the upper region of the body alone. Shielding of the head greatly reduces the damage done to the spleen, although the spleen is exposed. These findings are consistent with the suggestion that the directly observable spleen damage is moderated by radiation-induced imbalance (Benjamin and Yost, 1960). This is not to suggest that "direct" effects of radiation do not occur, as is indicated by the data obtained with head-shielded Wistar rats. However, the shielding techniques used in this study permit a low level of radiation to reach the shielded areas, and thus the magnitude of direct effects is difficult to ascertain. Studies of this problem using x-radiation and complete shielding are in process.

Present data do not permit any conclusions about the cause of the radiation damage. The indirect effect of radiation is first detectable by our methods about 8 hours after exposure. The lag between exposure and effect is consistent with the postulate that the primary cause is an induced hormonal imbalance. It has been shown that the pituitary has released thyrotropic hormone one hour after irradiation (Mateyko and Edelmann, 1954) and that the thyroid follicles have been emptied after two hours (Botkin, Praytor, Austing and Jensen, 1952). Histological examination of these glands in this laboratory indicates the same pattern of hormonal release and indicates that such release precedes the observed depression of oxidative phosphorylation by several hours. On the other hand, histological examinations show that a number of cells have already succumbed to the effect of radiation during the first 8 hours after exposure. Consequently, either damage to the phosphorylating mechanism does not cause cell death or the uncoupling of phosphorylation in certain cells precedes that in others. In the latter case a small number of sensitive cells would go undetected by any present assay methods, since the entire cell population from several spleens is used for a single determination. Therefore, if we are to adopt the interpretation that

uncoupling of oxidative phosphorylation is a general effect of radiation which is directly or indirectly related to the cause of cell death, it must be assumed that the appearance of a physiological change at 8 hours means that the damage has become *extensive* by that time. Clearly, the data are not sufficient to permit any final conclusion; on the other hand, the indirect effect of the radiation (as seen with shielded spleens) indicates that the hormonal imbalance hypothesis may still be applicable to cell death.

In all cases, recovery of oxidative phosphorylation precedes histological recovery (compare Tables I and II with V and VI). Of course, the data only permit the correlation of the recovery of phosphorylation in the surviving cells of the spleen with the regeneration of certain areas of the spleen whose phosphorylative ability cannot be measured by any present techniques. Since correlations seldom permit conclusions about causality, the data merely indicate the state of the general background against which other processes in the organism are taking place. Although it is attractive to suggest that the recovery of the phosphorylating mechanism is responsible for regenerative processes, it is equally possible that the uncoupling of phosphorylation is a general stress response which permits a more rapid general metabolism required to provide various carbon skeletons for the synthesis of new cellular components. Until it is possible to separate repair processes from recovery of phosphorylation, no critical test of these various hypotheses will be available.

Perhaps the most important point which can be drawn from these data is that protective effects of shielding are protective of the entire recovery mechanism. As Table VII shows, the recovery of liver phosphorylation can be accelerated by shielding of parts other than the liver. Although the acceleration of recovery in the liver is slight, it is nonetheless significant. The standard deviation of the 6-day runs is 0.08 and of the 8-day runs is 0.16. It seems unlikely that in the liver this effect is brought on by a "repopulation of cells from lymphocyte tissue." Rather, the general changes in the organism which occur during the recovery period must have a widespread effect in the various organs of the body.

Although the results presented here are in general agreement with previous work on the inactivation of the phosphorylating mechanism (van Bekkum, 1957; Benjamin and Yost, 1960) and with the increased survival of irradiated rats having part of their body shielded (Jacobson, 1954), it should be noted that the recovery time is much longer than that given previously by Benjamin and Yost (1960). No simple reason can be given for this difference nor for the difference between Wistar and Sprague-Dawley rats. It is probable that the methods previously used to assay P:O ratios were not completely satisfactory, as suggested by Thomson (1964). However, the type of indirect effect observed in these studies depends upon a number of environmental factors and thus can be controlled only with difficulty. A discussion of some of these problems will be presented elsewhere, as it has little bearing on the essential points discussed in this paper.

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SUMMARY

White male rats of the Sprague-Dawley and Wistar strains were exposed to 800 r total-body and partial-body γ -radiation. Estimates of the damage to the oxidative phosphorylation mechanism of spleen, liver and testis of the Sprague-Dawley rats and of the spleen of Wistar rats were made. Similarly, changes in the histology of the spleen follicles were followed, as well as alterations in the circulating lymphocyte population. In general, there seems to be no indication of an initial resistance to radiation; however, there are obvious differences in the recovery rate between the strains and between the total-body and partial-body irradiated animals. Changes in oxidative phosphorylation closely parallel the histological changes and thus can be used as a rapid technique for accessing indirect damage. The data suggest that "radiation resistance" is more a matter of recovery rate than of initial resistance.

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